(19) Europäisches Patentamt
European Pat nt Office
Office urop é n des brevets

(11) Publication numb r : **0 666 270 A2** B1

(12) # EUROPEAN PATENT APPLICATION

(21) Application number : **95300736.6**

(51) Int. Cl.^{6} : **C07K 7/04**, C07K 14/47, A61K 38/02

(22) Date of filing : **07.02.95**

A request for correction of the sequence listing has been filed pursuant to Rule 88 EPC. A decision on the request will be taken during the proceedings before the Examining Division (Guidelines for Examination in the EPO, A-V, 3.).

(30) Priority : **08.02.94 US 193977**

(43) Date of publication of application : **09.08.95 Bulletin 95/32**

(84) Designated Contracting States : **AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE**

(71) Applicant : **Bristol-Myers Squibb Company**
345 Park Avenue
New York, N.Y. 10154 (US)

(72) Inventor : **Webster, Kevin R.**
804 Roelofs Road
Yardley, PA 19067 (US)
Inventor : **Coleman, Kevin G.**
29 Blackwell Avenue
Hopewell, NJ 08525 (US)

(74) Representative : **Jones, Alan John et al**
CARPMAELS & RANSFORD
43 Bloomsbury Square
London, WC1A 2RA (GB)

(54) **Peptide inhibitors of the p33cdk2 and p34cdc2 cell cycle regulatory kinases and human papillomavirus E7 oncoprotein.**

(57) Novel peptide and peptide mimetic ligands which act as inhibitors of p34^{cdc2} kinase, p33^{cdk2} kinase and human papillomavirus transforming protein E7 (HPV E7) are disclosed. The inhibitors are derived from the binding domains of a retinoblastoma tumor suppressor protein (Rb), p107 and a cyclin.

EP 0 666 270 A2

Technical Field

Th present invention relat s gen rally to peptide inhibitors of cell growth and proliferation. More particularly, th inv ntion relates to ligands which bind to p34^{cdc2}/p33^{cdk2} kinas and inhibit the activity thereof, and which inhibit the specific interaction between retinoblastoma tumor suppressor protein and human papillomavirus transforming protein E7.

Background of the Invention

Cancer cells are characterized by their ability to proliferate in a continuous, uncontrolled fashion. It is now clear that primary cell cycle regulators must be circumvented or directly involved in oncogenesis in order for this to occur.

Cell cycle regulation occurs at the boundaries of the G_1/S and G_2/M phases, two major transition points of the cell cycle. A key regulator of these transitions is p34^{cdc2} kinase which is known to phosphorylate a number of proteins including histone H1, DNA polymerase α, RNA polymerase II, retinoblastoma tumor suppressor protein (Rb), p53, nucleolin, cAb1, SV40 large T antigen and lamin A. For example, p34^{cdc2} kinase activity is required for entry of cells into mitosis, i.e., for passage from the G_2 phase of the cell cycle into the M phase. (Lee et al. (1988) *Trends Genet.* 4:289-90; Dunphy et al. (1988) *Cell* 54:423-431; Gautier et al. (1988) *Cell* 54:433-439; *see, for review,* Cross et al. (1989) *Ann. Rev. Cell Biol.* 5:341-395; Hunter (1989) *Curr. Opinion Cell Biol.* 1:268-274; Nurse (1990) *Nature* 344:503-508). The activity of p34^{cdc2} kinase is, in turn, regulated by both protein:protein interactions and post-translational modifications. Thus, blockage of either of these mechanisms leads to arrest of the mammalian cell cycle. For example, microinjection of p34^{cdc2} antibodies into serum-stimulated rat fibroblasts causes cells to arrest in G_2 and treatment of activated T lymphocytes with p34^{cdc2} antisense oligodeoxynucleotides inhibits DNA synthesis (Furukawa et al. (1990) *Science* 250:805-808; Riabowol et al. (1989) *Cell* 57:393-401). Injection of suc1 protein (a ligand of p34^{cdc2}) into HeLa cells arrests cell growth, presumably by disrupting normal p34^{cdc2} protein:protein interactions (Draetta (1990) *TIBS* 15:378-383). In addition, inhibition of the cdc25 phosphatase with specific antibodies blocks the post-translational modification of p34^{cdc2} and leads to HeLa cell death (Galaktionov et al. (1991) *Cell* 67:1181-1194). Rb, p107 protein and the cyclin (cyc) protein family have been shown to associate with p34^{cdc2} (and its homolog p33^{cdk2}) (Pines et al.1990) *Nature* 346:760-763; Tsai et al. (1991) *Nature* 353:174-177; Giordano et al. (1989) *Cell* 58:981-990) ; the E2F transcription factor; the adenovirus E1A protein; and the human papillomavirus transforming protein E7 (Whyte et al. (1988) *Nature* 334:124-129; Chelappan et al. (1991) *Cell* 65:1053-1061; Bandara et al. (1991) *Nature* 352:249-252; Mundryj et al. (1991) *Cell* 65:1243-1253; Pines et al. (1990), *supra*; Tsai et al. (1991), *supra*; Giordano et al. (1989), *supra*; Shirodkar et al. (1992) *Cell* 68:157-166; Devoto et al. (1992) *Cell* 68:157-166; DeCaprio et al. (1988) *Cell* 54:275-283; Dyson et al. (1989) *Science* 243:934-937; Gage et al. (1990) *J. Virol.* 64:723-730). The binding of cyclins to p34^{cdc2} or p33 ^{cdk2} is required for kinase activity (Solomon et al. (1990) *Cell* 63:1013-1024; Pines et al. (1990), *supra*; Tsai et al. (1991), *supra*; Giordano et al. (1989), *supra*).

The functional domains of the Rb and p107 proteins have been mapped through both genetic and biochemical means. (Hu et al. (1990) *EMBO J.* 9:1147-1155; Ewen et al. (1991) *Cell* 66:1155-1164; Ewen et al. (1992) Science 255:85-87). An approximately 400 amino acid fragment of Rb and p107, termed the Rb pocket, is responsible for association of these proteins with the DNA tumor virus oncoproteins and cellular ligands. Within this domain are six regions of extensive sequence similarity between Rb and p107. (Ewen et al. (1991), *supra*). Likewise, the cyclins share a large region of sequence similarity spanning approximately 87 amino acids, which has been designated the "cyclin box." (Pines et al. (1989) *Cell* 58:833-846) This domain is thought to play a role in protein:protein interactions and it has been shown that deletion of sequences amino-terminal to this domain do not affect cyclin function. (Murray et al. (1989) *Nature* 339:280-286; Lew et al. (1991) *Cell* 66:1197-1206).

p34^{cdc2} protein:protein interactions are altered in human tumors. For example, the gene encoding the cofactor cyclin A is disrupted in hepatocellular carcinoma (Wang et al. (1990) *Nature* 343:555-557). Also, recent data have demonstrated that cyclin D1 (PRAD1) is within the *bcl-1* locus and is rearranged in parathyroid tumors and some B cell leukemias (de Boer et al. (1993) *Cancer Res.* 53:4148-4152; Motokura et al. (1991) *Nature* 350:512-515). In addition, the *bcl-1* locus is frequently amplified in breast carcinoma and cyclin D1 is overexpressed in mouse skin carcinoma (Lammie et al. (1991) *Oncogene* 6:439-444; Bianchi et al. (1993) *Oncogene* 8:1127-1133; Buckley et al. (1993) *Oncogene* 8:2127-2133). Furthermore, the subunits of the cdk kinases are rearranged in transformed cells wh n compared to their normal counterpart (Xiong et al. (1993) *Genes and Development* 7:1572-1583). This is the result of the loss or underexpression of waf1/cip1 protein, which is normally a repressor of cdk kinase activities, and is regulated by the p53 tumor suppressor protein (Xiong et al. (1993) *Nature* 366:701-704; Serrano et al. (1993) *Nature* 366:704-707; Gu et al. (1993) *Nature* 366:707-710;

Harp r et al. (1993) *Cell* 75:805-816; El-Deiry et al. (1993) *Cell* 75:817-825). These data clearly implicate the alteration of $p34^{cdc2}$ kinase activity in oncogenesis.

Accordingly, inhibitors of $p34^{cdc2}$ activity would b useful in th regulation and control of the continuous, prolif rative growth of cancerous cells.

Summary of the Invention

The present invention provides for ligands that inhibit the course of cellular proliferation by preventing progression through the G_1/S or G_2/M boundaries by inhibition of $p34^{cdc2}$ kinase activity. The ligands are useful as modulators of $p34^{cdc2}$ kinase-mediated cell growth and proliferation and, thus, as agents for studying the mechanisms by which cancer cells proliferate in a continuous fashion and as agents for ameliorating tumors associated with disruption of genes which code for essential cyclins, for example, hepatocellular carcinomas, parathyroid tumors, various B cell leukemias and breast carcinomas or tumors which contain a p53 mutation or loss of waf1/cip1.

Accordingly, in one embodiment, the invention is directed to an inhibitor of $p34^{cdc2}$ cell cycle regulatory kinase activity, or an inhibitor of the kinase activity of a homolog thereof, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin.

In preferred embodiments, the inhibitor is a peptide including the amino acid sequence LCAFYIMAK [SEQ ID NO:________________], a peptide including the amino acid sequence MCSMYGICK [SEQ ID NO:________________], a peptide including the amino acid sequence CAFYI [SEQ ID No:________________], or substitutions of these sequences which retain inhibitory activity.

In a further embodiment, the invention is directed to an inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin.

In another preferred embodiment, the invention is directed to a complex comprising:

(a) $p34^{cdc2}$ or a homolog thereof; and

(b) an inhibitor of $p34^{cdc2}$ cell cycle regulatory kinase activity, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin, and wherein the complex substantially lacks kinase activity.

In another embodiment, the invention is directed to a complex comprising:

(a) human papillomavirus E7 protein; and

(b) an inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of an Rb, p107 and a cyclin.

In a further embodiment, the invention is directed to a method of inhibiting $p34^{cdc2}$ cell cycle regulatory kinase activity, or the kinase activity of a homolog thereof, comprising providing a $p34^{cdc2}$ inhibitor as described above and contacting $p34^{cdc2}$ or the homolog thereof with an inhibiting amount thereof.

In an additional embodiment, the invention is directed to a method of inhibiting human papillomavirus E7 activity, comprising providing a human papillomavirus E7 inhibitor and contacting human papillomavirus E7 with an inhibiting amount thereof.

These and other embodiments of the subject invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Figures

Figure 1 depicts a comparison of the amino acid sequences of various $p34^{cdc2}$ binding proteins including cyclins A, B1, C, D and E, p107 and Rb [SEQ ID NOS:________________]. Gaps were introduced to maximize homology, and are represented by dashes. Amino acids are represented by their single letter code. Boxes indicate identical amino acids or conservative changes. The stipled cylinder represents the α-helix II region.

Figure 2 depicts a plot of the percent inhibition of $p34^{cdc2}$ histone H1 kinase activity versus the p107 9mer peptide concentration.

Figure 3 depicts plots of the binding of the p107 30mer peptide to HPV18-E7 (Figure 3A: MBP-E7 (●), MBP alone (Δ)) or competition for the E7-p107 30mer peptide interaction with the p107 9mer peptide (LCAFYIMAK [SEQ ID NO:________________]) (Figure 3B).

Figure 4 depicts plots of the effect of p107 9mer peptide (LCAFYIMAK [SEQ ID NO:________________]) on the binding of MBP-E7 protein to Rb protein as determin d by ELISA (Figure 4A: MBP-E7 (○); MBP alone (Δ)) or by binding competition (Figure 4B: Rb bound (●)).

Detailed Description

Th practice of the present invention will mploy, unless otherwis indicated, conventional techniques of protein chemistry and biochemistry, mol cular biology, microbiology and r combinant DNA t chnology, which are within the skill of the art. Such techniques are explained fully in the literature. *See, e.g.,* Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual,* Second Edition (1989); *DNA Cloning,* Vols. I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.K. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL press, 1986); Perbal, B., *A Practical Guide to Molecular Cloning* (1984); the series, *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.).

All patents, patent applications and publications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

A. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "$p34^{cdc2}$," "$p34^{cdc2}$ kinase" or "$p34^{cdc2}$ cell cycle regulatory kinase", which terms are used interchangeably, is meant an approximately 32-34 kda protein, alternatively known as MPF (maturation/M Phase promoting factor or mitosis-promoting factor), which possesses protein-serine/threonine kinase activity (as described in Lee et al. (1987), *supra*; Dunphy et al. (1988), *supra*; and Gautier et al. (1988), *supra*). The term encompasses the product of the *cdc2* gene of *Schizosaccharomyces pombe* and the product of the *CDC28* gene from *Saccharomyces cerevisiae*, and homologues found in other species (*see*, Arion et al. (1988) *Cell* 55:371-378; Dunphy et al. (1988), *supra*; Gautier et al. (1988), *supra*; and Labbé et al. (1988) *Cell* 57:253-263), including the human homolog of these proteins, $p34^{cdc2}$ (Lee et al. (1987), *supra*). $p34^{cdc2}$ kinase activity is dependent on association with specific cyclins and post-translational modification of Thr-14 and Tyr-15 amino acid residues.

The term "$p33^{cdk2}$," "$p33^{cdk2}$ kinase" or "$p33^{cdk2}$ cell cycle regulatory kinase" refers to a cyclin-dependent kinase homolog of $p34^{cdc2}$ kinase, the activity of which is similarly dependent on association with one or more cyclin molecules (Tsai et al. (1991), *supra*; Rosenblatt et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:2824-2828; Elleage et al. *Proc. Natl. Acad. Sci. USA* 89:2907-2911).

By "a $p34^{cdc2}$ binding domain" is meant that portion of the molecule in question (i.e., an Rb, p107 or a cyclin) which interacts either directly or indirectly with $p34^{cdc2}$ kinase thereby preventing the activation of the kinase. The binding domain may be a sequential portion of the molecule, i.e., a contiguous sequence of amino acids, or it may be conformational, i.e., a combination of non-contiguous sequences of amino acids which, when the molecule is in its native state, forms a structure which interacts with $p34^{cdc2}$ kinase.

By being "derived from" a binding domain is meant any molecular entity which is identical, substantially homologous, complementary or otherwise functionally or structurally equivalent to the native $p34^{cdc2}$ binding domain of the molecule in question (i.e., an Rb, p107 or a cyclin). Thus, a molecule derived from a particular binding domain may encompass the amino acid sequence of a naturally occurring ligand-binding site, any portion of that binding site, or other molecular entity that functions to bind to an associated ligand. A molecule derived from such a binding domain will interact either directly or indirectly with $p34^{cdc2}$ kinase in such a way as to mimic the native binding domain. Such molecular entities may include competitive inhibitors, peptide mimetics and the like.

An "inhibitor of $p34^{cdc2}$ kinase activity" or an "inhibitor of $p33^{cdk2}$ kinase activity" is intended to mean a peptide or peptide fragment derived from a $p34^{cdc2}$ binding domain of an Rb, p107 or a cyclin, which interacts either directly or indirectly with $p34^{cdc2}$ or $p33^{cdk2}$ and prevents activation of the kinase. If the inhibitor is a cyclin protein, it will not include the full-length sequence of the wild-type molecule. An inhibitor can prevent cyclin-dependent kinase activation by competitively inhibiting the binding of cyclins to $p34^{cdc2}$. In addition, peptide mimetics, synthetic molecules with physical structures designed to mimic structural features a particular peptide, may similarly serve as inhibitors of kinase activity. Such inhibitors diminish the $p34^{cdc2}$ or $p33^{cdk2}$ enzymatic catalysis of the transfer of the terminal phosphate of ATP, or a similar ATP analog or other nucleotide triphosphate, to a suitable substrate. Such substrates include histone H1, DNA polymerase α, RNA polymerase II, Rb, p53, nucleolin, cAb1, SV40 larg T antigen, lamin A and the lik . Alternatively, such inhibitors may diminish the ability of activated (e.g., dephosporylated) $p34^{cdc2}$ to support progression through the cell cycle as measured by a suitable cell cycle assay (*see, e.g.,* Pines et al. (1989), *supra*). Such inhibition may be by a direct, competitive mechanism, or by an indirect, non- or uncompetitive mechanism.

An "inhibitor of human papillomavirus E7 protein" is a molecule which interacts with transforming protein E7, either directly or indirectly, to prevent the formation of the specific complex with Rb. As with inhibitors of p34^{cdc2} kinase, th se inhibitors can prevent binding by competitiv inhibition, or by an indirect, non-or uncompetitive mechanism. In addition, the term encompasses p ptide inhibitors as well as p ptid mimetics.

The inhibitory activity of a candidate peptide or peptide mimetic can be tested by assessing the ability of the candidate to bind to the target acceptor, i.e., p34^{cdc2}, p33^{cdk2} or HPV E7. For example, those candidates which bind to the target acceptor with suitable affinity and specificity can be assayed for their ability to inhibit the activity of the target acceptor. For p34^{cdc2} and p33^{cdk2}, the ability of the inhibitor to diminish kinase activity can be assessed. For HPV E7, the ability of a candidate inhibitor to compete with Rb for binding to E7 can be determined. Finally, to assess the inhibitory activity in a whole cell setting, the ability of a candidate inhibitor to prevent the progression of a synchronized population of transformed cells through the cell cycle can be determined. For example, HeLa cells can be synchronized using techniques well-known in the art (*see*, Lew et al. (1991), *supra*). The inhibition of cell cycle progression through the G_1/S boundary is assessed by a diminution of DNA synthesis in the cell population. Inhibition of cell cycle progression through the G_2/M boundary is determined by the absence of mitotic activity (i.e., cell division).

"Peptide mimetics" are structures which serve as substitutes for peptides in interactions with acceptor molecules (*see* Morgan et al. (1989) *Ann. Reports Med. Chem.* 24:243-252 for a review of peptide mimetics). Peptide mimetics, as used herein, include synthetic structures which may or may not contain amino acids and/or peptide bonds, but retain the structural and functional features of a peptide ligand. The term, "peptide mimetics" also includes peptoids and oligopeptoids, which are peptides or oligomers of N-substituted amino acids (Simon et al. (1972) *Proc. Natl. Acad. Sci. USA* 89:9367-9371). Further included as peptide mimetics are peptide libraries, which are collections of peptides designed to be of a given amino acid length and representing all conceivable sequences of amino acids corresponding thereto. Methods for the production of peptide mimetics are described more fully below.

Two polypeptide sequences are "substantially homologous" when at least about 85% (preferably at least about 85% to 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule. As used herein, substantially homologous also refers to sequences showing identity to the specified polypeptide sequence.

The terms "polypeptide", "peptide" and "protein" are used interchangeably and refer to any polymer of amino acids (dipeptide or greater) linked through peptide bonds. Thus, the terms "polypeptide", "peptide" and "protein" include oligopeptides, protein fragments, analogues, muteins, fusion proteins and the like.

The following single-letter amino acid abbreviations are used throughout the text:

Alanine	A	Arginine	R
Asparagine	N	Aspartic acid	D
Cysteine	C	Glutamine	Q
Glutamic acid	E	Glycine	G
Histidine	H	Isoleucine	I
Leucine	L	Lysine	K
Methionine	M	Phenylalanine	F
Proline	P	Serine	S
Threonine	T	Tryptophan	W
Tyrosine	Y	Valine	V

B. General Methods

Central to the pres nt invention is the discovery of peptide molecules which bind to and inhibit p34^{cdc2} kinase activity. These molecules ar derived from th binding domain of an Rb, p107 or a cyclin. These proteins, depicted in Figure 1, bind p34^{cdc2}, its homolog p33^{cdk2}, the E2F transcription factor and the adenovirus E1A transforming protein. In addition, Rb forms a specific complex with the transforming protein of papillomavirus E7. Th peptide inhibitors, and peptide mimetics thereof, provide useful tools for the analysis of the normal

function of $p34^{cdc2}$ and $p33^{cdk2}$ kinases and transforming factors, such as E7, in cell growth and proliferation in precancerous and cancerous cells. Additionally, since the activity of $p34^{cdc2}$ kinase is essential for the progression through the G_1/S and G_2/M transition phases of the cell cycle, th protein inhibitors, or mimetics thereof, can b administered to cancerous tissu s in order to suppress tumor growth.

As shown in the examples, the claimed inhibitors of $p34^{cdc2}$ kinase, $p33^{cdk2}$ kinase or HPV E7 include sequences of amino acids derived from the binding domains of proteins which interact with $p34^{cdc2}$ and/or HPV E7, and mimetics thereof. In particular, the inhibitors are derived from a region of homology found in a number of $p34^{cdc2}$ binding proteins known as the α-helix II region, shown in Figure 1. A 30 amino acid peptide, FEFTLVHCPDLMKDLMKDRHLDWLLLCAFYIMAK, [SEQ ID NO:________________] encompassing the α-helix II sequence from p107 has been shown herein to bind to $p34^{cdc2}$ and $p33^{cdk2}$. This 30 amino acid peptide also inhibits the histone H1 kinase activity of $p34^{cdc2}$ and $p33^{cdk2}$. By sequentially deleting amino- or carboxy-terminal amino acids, a five amino acid sequence (CAFYI [SEQ ID NO:________________]) was identified as a minimum sequence which retains inhibitory activity. Furthermore, a peptide having nine amino acids (LCAFYIMAK [SEQ ID NO:________________]), including the 5 amino acid peptide, was shown to bind to $p34^{cdc2}$ with an affinity of approximately 10 μM. Thus, useful $p34^{cdc2}$ inhibitors of the present invention will be derived from at least this 5mer, and can include as many as 50 to 200 or more amino acids, so long as a proper binding conformation is retained.

The above-described 30mer peptide also binds to human papillomavirus (HPV) 18-E7 protein. Furthermore, the 30mer peptide inhibits E7 binding to Rb, as does a nine amino acid peptide derived from Rb (MCSMYGICK [SEQ ID NO:________________]).

As explained above, all of these peptides, as well as molecules substantially homologous, complementary or otherwise functionally or structurally equivalent to these peptides, may be used for purposes of the present invention.

The peptide inhibitors of the present invention may be synthesized by conventional techniques known in the art, for example, by chemical synthesis such as solid phase peptide synthesis. Such methods are known to those skilled in the art. In general, these methods employ either solid or solution phase synthesis methods, well known in the art. *See, e.g.,* J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis,* 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology,* editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis,* Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology, supra,* Vol. 1, for classical solution synthesis.

As explained above, peptide mimetics which structurally and functionally mimic the peptide inhibitors described above will also find use herein and may be generated using the following strategies and procedures. Generally, mimetics are designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chain moieties by a methyl group or pseudoisosteric groups with different electronic properties (*see* Hruby et al. (1990) *Biochem. J.* 268:249-262), and by systematic replacement of peptide bonds in the above described peptide inhibitors with amide bond replacements. For example, analogues containing amide bond surrogates may be used to investigate aspects of peptide structure and function, such as rotational freedom in the backbone, intra- and intermolecular hydrogen-bond patterns, modifications of local and total polarity and hydrophobicity, and oral bioavailability.

Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic inhibitor of $p34^{cdc2}$, $p33^{cdk2}$, HPV E7 or other acceptor. For example, β,β-disubstituted amino acids may be used to examine the effects of conformational constraints on peptide activity (*see, e.g.,* Manning et al. (1982) *J. Med. Chem.* 25:408-414; Mosberg et al. (1983) *Proc. Natl. Acad. Sci. USA* 106:506-512; Pelton et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:236-239).

The mimetics can include isosteric amide bonds such as $\Psi[CH_2S]$, $\Psi[CH_2NH]$, $\Psi[CSNH_2]$, $\Psi[NHCO]$, Ψ-$[COCH_2]$ and Ψ[(E) or (Z) CH=CH] (*see, for review,* Spatola (1983) in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Volume VII, (Weinstein, ed.), Marcel Dekker, New York, 267-357). Structures which mimic the tetrahedral transition state associated with hydrolysis of a substrate bond can also be present and include hydroxymethylene, fluoroketone moieties and phosphoramidate transition state mimics (Bühlmayer et al. (1988) *J. Med. Chem.* 31:1839; Sham et al. (1988) *FEBS Lett.* 220:299; Matthews (1988) *Acc. Chem. Res* 21:333). The synthetic molecules can also include D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule from enzymatic degradation (*see, e.g.,* Freidinger et al. (1985) in "Peptides: Structure and Function," (Deber et al., ds.), Pierce Chem. Co., Rockford, IL, 549-552; Sawyer et al. (1980) *Proc. Natl. Acad. Sci. USA* 77:5754-5758; Torchiana et al. (1978) *Arch. Int. Pharmacol. Ther.* 235:170-176). Cyclic amino acid analogues may b used to constrain amino acid residues to particular conformational stat s, e.g., αα'-and ββ'-substituted cyclic amino acids such as l-aminocyclopentanecarboxylic

acid (cycloleucine) and β,β-cyclopentamethylene-β-mercaptopropionic acid (*see* Hruby et al. (1990), *supra*).

Th mimetics can also include mimics of inhibitor peptide secondary structure -- structures which can mod l th 3-dimensional orientation of amino acid residu s into the known s condary conformations of proteins -- including β-turn mimetics, such as phenoxathin ring system, and β-sh t mimics, such as epindolidione structures. Design, synthesis and conformational analysis of an α-helix inducing template has been described (Kemp et al. (1988) *Tetrahedron Lett.* 29:4931; Kemp et al. (1988) *Tetrahedron Lett.* 29:4935).

Similarly, peptoids will find use herein. Peptoids are oligomers of N-substituted amino acids (Simon et al. (1972), *supra*), and can be used as motifs for the generation of chemically diverse libraries of novel molecules, which can then be tested for binding and inhibitory activity against $p34^{cdc2}$, $p34^{cdk2}$, HPV E7 or other acceptor molecules. The monomers may incorporate *t*-butyl-based side-chain and 9-fluorenylmethoxy-carbonyl α-amine protection. Oligomerization of the peptoid monomers may be performed by, for example, *in situ* activation by either benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorphosphate or bromotris(pyrrolidino)phosphonium hexafluorophosphate. Other steps are identical to conventional peptide synthesis using α-(9-fluorenylmethoxycarbonyl)amino acids. Oligopeptoids may be identified which have affinities comparable to the corresponding inhibitory peptides and, thus, are useful in $p34^{cdc2}$ and $p33^{cdk2}$ kinase or HPV E7 binding assays (*see* Simon et al. (1992), *supra*).

Peptide ligands that interact with $p34^{cdc2}$, $p33^{cdk2}$, HPV E7 or other protein acceptors can be developed by using a biological expression system (*see* Christian et al. (1992) *J. Mol. Biol.* 227:711-8; Devlin et al. (1990) *Science* 249:404-406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382). The use of such systems allows the production of large libraries of random peptide sequences and the screening of these libraries for peptide sequences that bind to particular proteins. The libraries may be produced by cloning synthetic DNA that encodes random peptide sequences into *Escherichia coli* expression vectors. In the filamentous phage system, foreign peptide sequences can be expressed on the surface of the infectious phage (*see* Smith (1985) *Science* 228:1315-1317; Parmley et al. (1988) *Gene* 73:305-318).

For example, a library may be made by ligating into an appropriate phage, a synthetic DNA fragment containing a degenerate coding sequence$(NNK)_n$, where N stands for an equal mixture of the deoxynucleotides G, A, T, and C, K stands for an equimolar mixture of G and T, and n stands for the number of amino acid residues desired in the product peptide. Affinity purification of phage displaying affector-binding peptides may be done by biotinylating the affector, incubating the phage with the biotinylated affector and reacting the phage on streptavidin-coated plates. Bound phage are eluted and amplified on agar medium and subjected to further rounds of affinity purification. Phage from later rounds of affinity purification are cloned and propagated, their DNAs sequenced to determine the amino acid sequences of their expressed peptide and their binding to $p34^{cdc2}$, $p33^{cdk2}$, HPV E7 or other affector molecules assessed by enzyme-linked immunosorbent assays (ELISA). Such libraries consisting of large numbers of clones expressing different short peptide sequences can be used to map binding domains.

Large libraries of peptide inhibitors can also be constructed by concurrent synthesis of overlapping peptides as described in U.S. Patent No. 4,708,871 to Geysen. The synthetic peptides can be tested for interaction with acceptor molecules by ELISA while still attached to the support used for synthesis. The solid support is generally a polyethylene or polypropylene rod onto which is graft polymerized a vinyl monomer containing at least one functional group to produce polymeric chains on the carrier. The functional groups are reacted to provide primary or secondary amine groups which are sequentially reacted with amino acid residues in the appropriate order to build the desired synthetic peptide using conventional methods of solid phase peptide chemistry.

Once produced, the inhibitory peptides or peptide mimetics can be used in pharmaceutical compositions to ameliorate tumors associated with disruption of genes which code for essential cyclins, for example, hepatocellular carcinomas, parathyroid tumors, some B cell leukemias and certain breast carcinomas or tumors associated with the loss of p53 function. The inhibitory peptides of the present invention can be formulated into therapeutic compositions in a variety of dosage forms such as, but not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the particular cancer type targeted. The compositions also preferably include pharmaceutically acceptable vehicles, carriers or adjuvants, well known in the art, such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or th like, and combinations thereof. Actual methods of preparing such compositions are known, or will be apparent, to those skilled in the art. *See, e.g., Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 18th edition, 1990.

The above compositions can be administered using conventional modes of delivery including, but not lim-

ited to, intravenous, intraperiton al, oral, intralymphatic, or subcutaneous administration. Local administration, to the tumor in question, will also find use with the pr sent invention.

Therapeutically effectiv doses will be asily determined by one of skill in the art and will dep nd on the s verity and course of th disease, th patient's h alth and response to treatment, and th judgment of the treating physician.

C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Experimental Methods

HeLa Cell Lysates:

HeLa cell extracts served source of $p34^{cdc2}$ and $p33^{cdk2}$ and were prepared as follows. HeLa cells were lysed in 20 mM N-[2-hydroxyethyl]piperazine-N'-[2-hydroxypropanesulfonic acid] (HEPES), pH 7.0, 150 mM NaCl, 5 mM sodium vanadate, 10 mM $Na_4P_2O_7$, 1 mM $ZnCl_2$, 2 mM ethylene-diaminetetraacetic acid (EDTA), 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 0.25% (v:v) NP-40, 1 mM Benzamidine, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 20 µg/ml each: $N_2S_2O_3$, leupeptin, Antipan and Pepstatin, followed by centrifugation to remove cell debris.

Immunoprecipitation of $p34^{cdc2}$:

Immunoprecipitation of $p34^{cdc2}$ from HeLa cell extracts was performed using previously reported procedures (Harlow et al. (1988) in: *Antibodies: A Laboratory Manual*, ed. Harlow and Lane, Cold Spring Harbor: Cold Spring Harbor Laboratory, pp 421-470) and anti-$p34^{cdc2}$ antibody, G6 (Gibco, BRL). Immunoprecipitation by this method yields an activated kinase complex (Draetta et al. (1988), *Cell* 54:17-26).

Assay of $p34^{cdc2}$ Kinase Activity:

Histone H1 kinase reactions were performed in 50 mM Tris, pH 8.0, 10 mM $MgCl_2$, 0.5 mM DTT, 1 mM EGTA plus 0.1 mM ATP, 1 µCi γ-[^{32}P]-ATP and 10 µg histone H1 (Boeringer Mannheim), and a source of $p34^{cdc2}$. Reactions were incubated at 30°C for 20 minutes and stopped by the addition of protein gel sample buffer. Kinase reactions were separated by SDS-PAGE (12.5% gel). The bands were detected by autoradiography and the amount of ^{32}P incorporated was quantitated using an Ambis Radioanalytic Imaging System.

Example 1

Comparison of $p34^{cdc2}$ Binding Proteins

Inhibitors of $p34^{cdc2}$ cell cycle regulatory kinase activity were identified by first comparing the primary amino acid sequence of known $p34^{cdc2}$-associated proteins: human cyclins A, B1, C, D, E, p107 protein and retinoblastoma tumor suppressor protein (Rb) [SEQ ID NOS:________________]. The basic alignment of the human cyclin proteins was demonstrated by Lew et al. (1991), *supra*, and the alignment of Rb and p107 was performed by Ewen et al. (1991), *supra*. The sequences of all seven proteins was subsequently compared, as depicted in Figure 1. Secondary structure analysis of all seven sequences was performed using both Chou-Fasman (Chou et al. (1974) *Biochemistry* 13:211-222; Chou et al. (1974) *Biochemistry* 13:222-245) and Robson-Garnier (Garnier et al. (1978) *J. Mol. Biol.* 120:97-120) structure prediction programs. Two regions of common α-helical structure, designated α-helix I and α-helix II, were predicted for six of seven proteins by both structure prediction programs.

A 170 amino acid region of sequence similarity was observed among Rb, p107 and the human cyclins. Rb and p107 share the highest degree of similarity with cyclin E, 27% and 34%, respectively. These sequences contain two regions of common pr dicted α-helical structure. Projection of the α-helix II sequences of Rb, p107 and cyclin E on a helical wh el demonstrates that the conserved residues are displayed on one face of th α-

helix.

Exampl 2

Binding of $p34^{cdc2}$ to p107.

In order to test whether the α-helix II domain is involved in the interaction of Rb, p107 and cyclin E with $p34^{cdc2}$, a 30 amino acid peptide from p107 (residues 692-721: FEFTLVHCPDLMKDLMKDRHLDWLLLCAFYI-MAK [SEQ ID NO: ________]) encompassing α-helix II, was synthesized and assayed for its ability to associate with $p34^{cdc2}$. The 30mer was covalently linked to CH-activated sepharose 4B (Pharmacia) as follows. The p107 30 amino acid peptide, which was insoluble in aqueous solutions, was linked to the sepharose in N,N-dimethylformamide. The concentration of 30mer linked to the bead was 0.85 μmole/ml as determined by HPLC. The p107 30mer-sepharose was incubated with total HeLa cell lysate and washed with 25 volumes of lysis buffer. The bound proteins were eluted with increasing concentrations of NaCl, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12.5% gel) and transferred to polyvinylidene difluoride (PVDF) membranes. $p34^{cdc2}$ was detected by probing the membrane with anti-$p34^{cdc2}$ antibody, G6 (Gibco, BRL) (*see*, Draetta et al. (1988), *supra*).

The p107 30mer-sepharose binds to $p34^{cdc2}$ and the majority of the complex was stable to 2M NaCl. These results indicate that the amino acid sequence within α-helix II contains sufficient information to direct interaction with $p34^{cdc2}$.

Example 3

Binding of $p33^{cdk2}$ to p107

The ability of p107 to bind to $p33^{cdk2}$ was examined using the methods described in Example 2. p107 30mer-sepharose beads were incubated with HeLa cell extract (3 x 10^5 cells per reaction) and then washed extensively. Bound complexes were then separated on a 12.5% SDS-PAGE gel and blotted onto a PVDF membrane. The blot was then probed with the kinase antibody. The results of this western blot analysis with anti-$p33^{cdk2}$ antibody (Upstate Biotechnology) indicates that the p107 30 amino acid peptide binds this kinase subunit.

Example 4

Histone H1 Kinase Activity of Bound and Immunoprecipitated $p34^{cdc2}$.

In order to examine the effect of binding of $p34^{cdc2}$ to p107 30mer-sepharose on $p34^{cdc2}$ kinase activity, histone H1 kinase activities associated with p107 30mer-sepharose and immunoprecipitated $p34^{cdc2}$ were compared as follows. $p34^{cdc2}$ was bound to p107 30mer-sepharose as described in Example 2. Histone H1 kinase reactions were performed using either immunoprecipitated or p107 30mer-sepharose bound $p34^{cdc2}$. The amount of $p34^{cdc2}$ bound to p107 30mer-sepharose was compared with that contained within the immune complex by western blot. Two times the amount of immunoprecipitated $p34^{cdc2}$ used in the kinase reaction was blotted to facilitate easier detection. The amount of $p34^{cdc2}$ associated with the p107 30mer-sepharose was determined by densitometry to be $\geqq$ 50 time greater than contained within the immune complexes. This data was used in normalizing the kinase activity associated with the different beads.

The observed histone H1 kinase activity for the p107 30 amino acid peptide-bound enzyme is similar to that of the immune complex. Normalization for the amount of $p34^{cdc2}$ protein present in both reactions demonstrated that the peptide bound enzyme has a specific activity equal to only 1.4% the specific activity of the immune complex. This is consistent with p107 30mer competing for cyclin binding to $p34^{cdc2}$, and is indicative of a common mechanism of binding for the cyclins, Rb, and p107.

Example 5

P ptid Inhibition of $p34^{cdc2}$

Histone H1 Kinase Activity

As a further test of the association of p107 with $p34^{cdc2}$, and to defin the minimal sequenc necessary

for interaction with $p34^{cdc2}$, th effect of p107 peptides on the $p34^{cdc2}$ immune complex histone H1 kinase activity was assessed. Both amino- and carboxy-terminal deletions were introduced into th original 30 amino acid peptide (*se* Tabl 1). Th peptides were synthesized by standard methods and purified by HPLC. Immune complex s were formed as d scribed in Example 4 and resuspended in kinas buffer plus 50 μM of the respective peptide solubilized in N,N-dimethylformamide, or an equal volume of solvent. After incubating the reactions for 15 minutes on ice, histone H1 and γ-[^{32}P]-ATP were added and the kinase assays were performed and quantitated as described in Example 4.

Addition of the p107 peptides inhibits histone H1 kinase activity associated with the immune complex (*see* Table 1). Addition of the p107 peptides to a $p33^{cdk2}$ kinase reaction also inhibits the activity of the kinase.

Peptides with deletions of the amino-terminal sequences to Cys_{713} and carboxy-terminal sequences to Ile_{717} retain the ability to inhibit $p34^{cdc2}$ activity. Further deletions to either Ala_{714} or Tyr_{716} resulted in inactive peptides. An unrelated 19mer control peptide had no effect upon histone H1 activity.

These data demonstrate that the minimal sequence which retained inhibitory activity was a pentapeptide with the sequence CAFYI [SEQ ID NO:________________]. Non-conservative amino acid substitutions within the pentapeptide totally abrogated $p34^{cdc2}$ kinase inhibition except at Cys_{713}.

Example 6

Effect of Single Amino Acid Substitution on $p34^{cdc2}$ Inhibitory Activity of p107 Peptides

In order to examine the role for Cys_{706} of Rb and Cys_{713} of p107 in the binding of $p34^{cdc2}$ and other ligands such as E1A or E2F, a peptide was synthesized which contained the Cys to Phe amino acid substitution at position 713 and its inhibitory activity was examined. This single mutation resulted in a 30% decrease in the peptide's ability to inhibit histone H1 kinase activity, confirming the importance of Cys_{713} in the p107 peptide-$p34^{cdc2}$ interaction (*see* Table 1, 9mer versus 713CF. This residue is also conserved in cyclins D and E and may function in a similar manner. In addition, the substitution of Ala for Tyr_{716}, which is highly conserved between the other proteins examined, totally abrogated $p34^{cdc2}$ kinase inhibition (*see* Table 1, 9mer versus 716YA. These data also suggest that the p107 peptides are capable of competing for and displacing cellular factors bound to $p34^{cdc2}$, which are required for kinase activation (Pines et al. (1989), *supra*; Murray et al. (1989), *supra*; Solomon et al. (1990), *supra*; Draetta et al. (1989) *Cell* 56:829-838) and are consistent with the earlier observation that low levels of kinase activity are associated with the p107 30mer peptide-sepharose.

TABLE 1				
		[SEQ ID NO.]	$p34^{cdc2}$ Inhib. (%)	IC_{50} (μM)
30mer	CAFYIMAK	---	ND	ND
21mer	DLMKDRHLDQLLLCAFYIMAK	---	70	ND
18mer	KDRHLDQLLLCAFYIMAK	---	80	ND
14mer	LDQLLLCAFYIMAK	---	80	ND
9mer	LCAFYIMAK	---	90	9
N7mer	AFYIMAK	---	0	ND
N5mer	YIMAK	---	0	>100
C7mer	LCAFYIM	---	70	ND
C6mer	LCAFYI	---	79	ND
C5mer	LCAFY	---	0	nd
NC5mer	CAFYI	---	82	13
NC4mer	CAFY	---	0	ND
713CF	LFAFYIMAK	---	60	15
714AE	LCEFYIMAK	---	0	23
715FA	LCAAYIMAK	---	0	>100
715FK	LCAKYIMAK	---	0	>100
715FY	LCAYYIMAK	---	20	45
716YA	LCAFAIMAK	---	0	40
716YF	LCAFFIMAK	---	80	25
717IK	LCAFYKMAK	---	0	>100
719AQ	LCAFYIMQK	---	75	ND
SV-NC5mer	PKKKRKVCAFYI	---	0	ND
RR-NC5mer	RRCAFYI	---	0	ND
PKG-NC5mer	RKRCAFYI	---	0	ND
SPKK-NC5mer	SPKKGCAFYI	---	0	ND
NC5mer-SPKK	CAFYIGSPKK	---	80	ND

Example 7

Affinity of the p107 9mer for 34^{cdc2} Assayed by Inhibition of Histone H1 Kinase Activity

The binding affinity of the 9mer peptide for $p34^{cdc2}$ was approximated by relating the percent inhibition of histone H1 kinase activity to peptide concentration. Reactions were performed as described in Example 4. The percent inhibition was quantitat d using an Ambis Radioanalytic Imaging System. The perc nt inhibition was plotted versus the peptide concentration. In calculating a binding constant, it was assumed that the concen-

tration of substrate and peptide were in vast excess of the p34^{cdc2} protein, which was assumed to be limiting in the reaction.

As depict d in Figure 2, th k_d was calculat d from the point of 50% inhibition, yi lding a $k_d \cong 10\ \mu M$. The binding affinity of the peptide for p34^{cdc2} may b similar to that of the whole molecul due to th ability of the peptide to efficiently inhibit p34^{cdc2} activity, which is directly related to its ability to compete with activating factors.

Example 8

Affinity of the p107 9mer for p34^{cdc2} Assayed by ELISA

An ELISA based assay was developed to measure the activity of p107 peptides and their derivatives. A 96-well cluster plate coated with the p107 30mer binds to p34^{cdc2} kinase from cell extracts. This binding is easily detectable with anti-p34^{cdc2} antibodies and can be used to quantitate the affinity of the p107 wild type and mutant peptides for the p34^{cdc2} kinase in addition to the kinase inhibition assay described in Example 7. A summary of all modifications to the original p107 peptides and their effect on binding and kinase inhibition is presented in Table 1. The affinity of the p107 9mer peptide for p34^{cdc2} kinase is approximately 10 μM as measured by the ELISA based competition assay, which is in agreement with the binding constant approximated from the kinase inhibition assay as described in Example 7.

Introduction of a non-conservative amino acid substitution within the pentapeptide sequence CAFYI [SEQ ID NO:________________] resulted in substantial decreases in both p34^{cdc2} inhibition and kinase binding except at Cys_{713}.

These data demonstrate that p107 protein is capable of associating with p34^{cdc2}. This is consistent with its homology to Rb and the cyclin proteins, and presents a structural basis for the functional similarities shared by these proteins.

Example 9

Inhibition of Human Papillomavirus-E7 Protein

Rb and p107 form specific complexes with the adenovirus E1A oncoprotein and Rb forms a similar complex with the E7 oncoprotein human papillomavirus (HPV). In order to determine whether the p107 30mer peptide domain and Rb may interact with other tumor suppressor proteins or other cellular and viral ligands, p107 30mer peptide binding to cellular and bacterially expressed human papillomavirus (HPV)-E7 protein was examined. p107 30 amino acid peptide-sepharose, prepared as described in Example 2, was incubated with CaSki cell (HPV-16 positive cervical carcinoma) extract and assayed for the binding of E7 protein. Western blot analysis demonstrated that the peptide did bind to HPV16-E7 protein from CaSki cells.

The association of bacterially expressed HPV18-E7 protein with the p107 30mer peptide was analyzed using an ELISA assay. In the experiment depicted in Figure 3A, a 96-well cluster plate was coated with p107 30mer peptide and incubated with increasing concentration of a MBP-E7 fusion protein or the MBP protein as a control. This experiment indicates that the p107 30mer peptide can bind directly to HPV-E7 protein and that binding is not dependent upon virus type. In the experiment depicted in Figure 3B, MBP-E7 fusion protein (100 ng) was incubated with a p107 30mer peptide-coated plate in the presence of increasing concentrations of free p107 9mer peptide. As shown in Figure 3, binding is directly proportional to the increase in the optical density at 405 nm. The results of competition experiments shown in Figure 3B indicate that the affinity of the p107 9mer peptide for the HPV18-E7 protein is approximately 1 nM, which is approximately 10,000-fold greater than for the p34^{cdc2} kinase.

Example 10

ELISA Assay of p107 Competition for E7-Rb Complex Formation

To examine the effect of p107 peptide binding upon the E7-Rb interaction, an ELISA assay was used to monitor E7-Rb complex formation. 96-w ll cluster plates were coated with either MBP-E7 protein (○) or MBP alone as a control (●). Th plates were incubated with increasing concentrations of baculovirus expressed Rb which was detected with monoclonal antibody αRb349 (Pharming n, San Diego CA) (Figure 4A). In a separate xperiment, 96-well cluster plat s wer coat d with MBP-E7 protein and incubated with 100 ng Rb in the presence of increasing concentrations of p107 9mer peptide (Figur 4B). Binding is quantitated as an increase in

optical density at 405 nm. The results of the experiments depicted in Figure 4A clearly demonstrate the ability of bacterially expressed E7-MBP fusion protein to bind to Rb. The results of the experiment shown in Figure 4B demonstrate that addition of increasing concentrations of p107 9m r p ptid to the reaction ffectively comp tes for E7 association with Rb. This r sult sugg sts that the p107 9mer peptid may be an eff ctiv inhibitor of HPV-E7 protein. A corresponding peptide from Rb protein (sequence MCSMYGICK [SEQ ID NO:________________]) also binds to the E7 oncoprotein and competes for the E7-Rb interaction.

Thus, inhibitors of $p34^{cdc2}$ and $p33^{cdk2}$, as well as inhibitors of human papillomavirus, have been disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: BRISTOL-MYERS SQUIBB COMPANY
(B) STREET: 345 PARK AVENUE
(C) CITY: NEW YORK
(D) STATE: NEW YORK
(E) COUNTRY: USA
(F) ZIP: 10154
(G) TELEPHONE: 206 727 3670
(H) TELEFAX: 206 727 3601

(ii) TITLE OF INVENTION: PEPTIDE INHIBITORS OF THE P33CDK2 AND P34CDC2 CELL CYCLE REGULATORY KINASES AND HUMAN PAPILLOMAVIRUS E7 ONCOPROTEIN

(iii) NUMBER OF SEQUENCES: 35

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Leu Cys Ala Phe Tyr Ile Met Ala Lys
1               5
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid

```
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

     Met Cys Ser Met Tyr Gly Ile Cys Lys
     1               5

(2) INFORMATION FOR SEQ ID NO:3:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 5 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

     Cys Ala Phe Tyr Ile
     1               5

(2) INFORMATION FOR SEQ ID NO:4:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 173 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

     Pro Asp Tyr His Glu Asp Ile His Thr Tyr Leu Arg Glu Met Glu Val
     1               5                   10                  15

     Lys Cys Lys Pro Lys Val Gly Tyr Met Lys Lys Gln Pro Asp Ile Thr
                 20                  25                  30

     Asn Ser Met Arg Ala Ile Leu Val Asp Trp Leu Val Glu Val Gly Glu
```

```
        35                  40                  45

Glu Tyr Lys Leu Gln Asn Glu Thr Leu His Leu Ala Val Asn Tyr Ile
    50                  55                  60

Asp Arg Phe Leu Ser Ser Met Ser Val Leu Arg Gly Lys Leu Gln Leu
65                  70                  75                  80

Val Gly Thr Ala Ala Met Leu Leu Ala Ser Lys Phe Glu Glu Ile Tyr
                85                  90                  95

Pro Pro Glu Val Ala Glu Phe Val Tyr Ile Thr Asp Asp Thr Tyr Thr
            100                 105                 110

Lys Lys Gln Val Leu Arg Met Glu His Leu Val Leu Lys Val Leu Thr
        115                 120                 125

Phe Asp Leu Ala Ala Pro Thr Val Asn Gln Phe Leu Thr Gln Tyr Phe
    130                 135                 140

Leu His Gln Gln Pro Ala Asn Cys Lys Val Glu Ser Leu Ala Met Phe
145                 150                 155                 160

Leu Gly Glu Leu Ser Leu Ile Asp Ala Asp Pro Tyr Leu
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 171 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Ser Glu Tyr Val Lys Asp Ile Tyr Ala Tyr Leu Arg Gln Leu Glu Glu
1               5                   10                  15

Glu Gln Ala Val Arg Pro Lys Tyr Leu Leu Gly Arg Glu Val Thr Gly
            20                  25                  30

Asn Met Arg Ala Ile Leu Ile Asp Trp Leu Val Gln Val Gln Met Lys
        35                  40                  45

Phe Arg Leu Leu Gln Glu Thr Met Tyr Met Thr Val Ser Ile Ile Asp
```

```
    50                  55                  60

Arg Phe Met Gln Asn Asn Cys Val Pro Lys Lys Met Leu Gln Leu Val
65                  70                  75                  80

Gly Val Thr Ala Met Phe Leu Ala Ser Lys Tyr Glu Glu Met Tyr Pro
                85                  90                  95

Pro Glu Ile Gly Asp Phe Ala Phe Val Thr Asp Asn Thr Tyr Thr Lys
            100                 105                 110

His Gln Ile Arg Gln Met Glu Met Lys Ile Leu Arg Ala Leu Asn Phe
        115                 120                 125

Gly Leu Gly Arg Pro Leu Pro Leu His Phe Leu Arg Arg Ala Ser Lys
    130                 135                 140

Ile Gly Glu Val Asp Val Glu Gln His Thr Leu Ala Lys Tyr Leu Met
145                 150                 155                 160

Glu Leu Thr Met Leu Asp Tyr Asp Met Val His
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 179 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Trp Gln Ser Ser His Tyr Leu Gln Trp Ile Leu Asp Lys Gln Asp Leu
1               5                   10                  15

Leu Lys Glu Arg Gln Lys Asp Leu Lys Phe Leu Ser Glu Glu Glu Tyr
            20                  25                  30

Trp Lys Leu Gln Ile Phe Phe Thr Asn Val Ile Gln Ala Leu Gly Glu
        35                  40                  45

His Leu Lys Leu Arg Gln Gln Val Ile Ala Thr Ala Thr Val Tyr Lys
    50                  55                  60

Arg Phe Tyr Ala Arg Tyr Ser Leu Lys Ser Ile Asp Pro Val Leu Met
```

```
65                  70                  75                  80

Ala Pro Thr Cys Val Phe Leu Ala Ser Lys Val Glu Glu Phe Gly Val
                85                  90                  95

Val Ser Asn Thr Arg Leu Ile Ala Ala Ala Thr Ser Val Leu Lys Thr
            100                 105                 110

Phe Ser Tyr Ala Phe Pro Lys Glu Phe Pro Tyr Arg Met Asn His Ile
        115                 120                 125

Leu Glu Cys Glu Phe Tyr Leu Leu Glu Leu Met Asp Cys Cys Leu Ile
    130                 135                 140

Val Tyr His Pro Tyr Arg Pro Leu Leu Gln Tyr Val Gln Asp Met Gly
145                 150                 155                 160

Gln Glu Asp Met Leu Leu Pro Leu Ala Trp Arg Ile Val Asn Asp Thr
                165                 170                 175

Tyr Arg Thr
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Asn Leu Leu Asn Asp Arg Val Leu Arg Ala Met Leu Lys Ala Glu Glu
1               5                   10                  15

Thr Cys Ala Pro Ser Val Ser Tyr Lys Cys Val Gln Lys Glu Val Leu
            20                  25                  30

Pro Ser Met Arg Lys Ile Val Ala Thr Trp Met Leu Glu Val Cys Glu
        35                  40                  45

Glu Gln Lys Lys Cys Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr
    50                  55                  60

Asp Arg Phe Leu Ser Leu Glu Pro Val Lys Lys Ser Arg Leu Gln Leu
```

```
65                   70                   75                   80

Leu Gly Ala Thr Cys Met Phe Val Ala Ser Lys Met Lys Glu Thr Ile
                85                  90                  95

Pro Leu Thr Ala Glu Lys Leu Cys Ile Tyr Thr Asp Asn Ser Ile Arg
            100                 105                 110

Pro Glu Glu Leu Leu Gln Met Glu Leu Leu Leu Val Asn Lys Leu Lys
        115                 120                 125

Trp Asn Leu Ala Ala Met Thr Ala His Asp Phe Ile Glu His Gly Leu
    130                 135                 140

Ser Lys Ile Ala Glu Ala Glu Glu Asn Lys Gln Ile Ile Arg Lys His
145                 150                 155                 160

Ala Gln Thr Phe Val Ala Leu Cys Ala Thr Asp Val Lys
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 174 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Trp Ala Asn Arg Glu Glu Val Trp Lys Ile Met Leu Asn Lys Glu Lys
1               5                   10                  15

Thr Tyr Leu Arg Asp Gln His Phe Leu Glu Gln His Pro Leu Leu Gln
            20                  25                  30

Pro Lys Met Arg Ala Ile Leu Leu Asp Trp Leu Met Glu Val Cys Glu
        35                  40                  45

Val Tyr Lys Leu His Arg Glu Thr Phe Tyr Leu Ala Gln Asp Phe Phe
    50                  55                  60

Asp Arg Tyr Met Ala Thr Gln Glu Asn Val Val Lys Thr Leu Leu Gln
65                  70                  75                  80

Leu Ile Gly Ile Ser Ser Leu Phe Ile Ala Ala Lys Leu Glu Glu Ile
```

```
                85                      90                      95

Tyr Pro Pro Lys Leu His Gln Phe Ala Tyr Val Thr Asp Gly Ala Cys
            100                 105                 110

Ser Gly Asp Glu Ile Leu Thr Met Glu Leu Met Ile Met Lys Ala Leu
        115                 120                 125

Lys Trp Arg Leu Ser Pro Leu Thr Ile Val Ser Trp Leu Asn Val Tyr
    130                 135                 140

Met Gln Val Ala Tyr Leu Asn Asp Leu His Glu Val Leu Leu Pro Gln
145                 150                 155                 160

Tyr Pro Gln Gln Ile Phe Ile Gln Ile Ala Glu Leu Leu Asp
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 168 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Val Arg Leu Arg Asp Leu Cys Leu Lys Leu Asp Val Ser Asn Glu Leu
1               5                   10                  15

Arg Arg Lys Ile Trp Thr Cys Phe Glu Phe Thr Leu Val His Cys Pro
            20                  25                  30

Asp Leu Met Lys Asp Arg His Leu Asp Gln Leu Leu Leu Cys Ala Phe
        35                  40                  45

Tyr Ile Met Ala Lys Val Thr Lys Glu Glu Arg Thr Phe Gln Glu Ile
    50                  55                  60

Met Lys Ser Tyr Arg Asn Gln Pro Gln Ala Asn Ser His Val Tyr Arg
65                  70                  75                  80

Ser Val Leu Leu Lys Ser Ile Pro Arg Glu Val Val Ala Tyr Asn Lys
                85                  90                  95

Asn Ile Asn Asp Asp Phe Glu Met Ile Asp Cys Asp Leu Glu Asp Ala
```

```
                    100                 105                 110

Thr Lys Thr Pro Asp Cys Ser Ser Gly Pro Val Lys Glu Glu Arg Ser
        115                 120                 125

Asp Leu Ile Lys Phe Tyr Asn Thr Ile Tyr Val Gly Arg Val Lys Ser
    130                 135                 140

Phe Ala Leu Lys Tyr Asp Leu Ala Asn Gln Asp His Met Met Asp Ala
145                 150                 155                 160

Pro Pro Leu Ser Pro Phe Pro His
                165
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Tyr Leu Arg Leu Asn Thr Leu Cys Glu Arg Leu Leu Ser Glu His Pro
1               5                   10                  15

Glu Leu Glu His Ile Ile Thr Leu Phe Gln His Thr Leu Gln Asn Glu
            20                  25                  30

Tyr Glu Leu Met Arg Asp Arg His Leu Asp Gln Ile Met Met Cys Ser
        35                  40                  45

Met Tyr Gly Ile Cys Lys Val Lys Asn Ile Asp Leu Lys Phe Lys Ile
    50                  55                  60

Ile Val Thr Ala Tyr Lys Asp Leu Pro His Ala Val Gln Glu Thr Phe
65                  70                  75                  80

Lys Arg Val Leu Ile Lys Glu Glu Glu Tyr Asp Ser Ile Ile Val Phe
                85                  90                  95

Tyr Asn Ser Val Phe Met Gln Arg Leu Lys Thr Asn Ile Leu Gln Tyr
            100                 105                 110

Arg Pro Pro Thr Leu Ser Pro Ile Pro His Ile Pro Arg Ser Pro Tyr
```

115 120 125

Lys Phe Pro Ser Ser Pro Leu Arg Ile Pro Gly Gly Asn Ile Tyr Ile
130 135 140

Ser Pro Leu Lys Ser Pro Tyr Lys Ile Ser Glu Gly Leu Pro Thr Pro
145 150 155 160

Thr Lys Met Thr Pro Arg Ile Leu Val Ser Ile Gly Glu
165 170

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Phe Glu Phe Thr Leu Val His Cys Pro Asp Leu Met Lys Asp Leu Met
1 5 10 15

Lys Asp Arg His Leu Asp Trp Leu Leu Leu Cys Ala Phe Tyr Ile Met
20 25 30

Ala Lys

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Phe Glu Phe Thr Leu Val His Cys Pro Asp Leu Met Lys Asp Arg His
1 5 10 15

Leu Asp Gln Leu Leu Leu Cys Ala Phe Tyr Ile Met Ala Lys
20 25 30

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Leu Met Lys Asp Arg His Leu Asp Gln Leu Leu Leu Cys Ala Phe
1 5 10 15

Tyr Ile Met Ala Lys
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Lys Asp Arg His Leu Asp Gln Leu Leu Leu Cys Ala Phe Tyr Ile Met
1 5 10 15

Ala Lys

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Asp Gln Leu Leu Leu Cys Ala Phe Tyr Ile Met Ala Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Phe Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Leu Cys Ala Phe Tyr Ile Met
1               5
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Leu Cys Ala Phe Tyr Ile
1               5
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Leu Cys Ala Phe Tyr
1               5
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Ala Phe Tyr
1

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Leu Phe Ala Phe Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Leu Cys Glu Phe Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Cys Ala Ala Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Leu Cys Ala Lys Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Leu Cys Ala Tyr Tyr Ile Met Ala Lys
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Leu Cys Ala Phe Ala Ile Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Leu Cys Ala Phe Phe Ile Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x Si)EQUENCE DESCRIPTION: SEQ ID NO:29:

Leu Cys Ala Phe Tyr Lys Met Ala Lys
1   5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Leu Cys Ala Phe Tyr Ile Met Gln Lys
1               5
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Pro Lys Lys Lys Arg Lys Val Cys Ala Phe Tyr Ile
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Arg Arg Cys Ala Phe Tyr Ile
1               5
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Arg Lys Arg Cys Ala Phe Tyr Ile
1               5
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Ser Pro Lys Lys Gly Cys Ala Phe Tyr Ile
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Cys Ala Phe Tyr Ile Gly Ser Pro Lys Lys
1               5                   10
```

Claims

1. An inhibitor of $p34^{cdc2}$ cell cycle r gulatory kinas activity, or an inhibitor of the kinase activity of a homolog th reof, wher in th inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein select d from the group consisting of a retinoblastoma protein, p107 and a cyclin.

2. The inhibitor according to claim 1, wherein the homolog is $p33^{cdk2}$.

3. An inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin.

4. The inhibitor according to any one of claims 1 to 3, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a retinoblastoma protein.

5. The inhibitor according to any one of claims 1 to 3, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of p107.

6. The inhibitor according to any one of claims 1 to 3, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of cyclin E.

7. The inhibitor according to claim 4, wherein the inhibitor is derived from the alpha-helix II domain of a retinoblastoma protein.

8. The inhibitor according to claim 5, wherein the inhibitor is derived from the alpha-helix II domain of p107.

9. The inhibitor according to claim 6, wherein the inhibitor is derived from the alpha-helix II domain of cyclin E.

10. The inhibitor according to claim 7, wherein the inhibitor comprises a peptide including the amino acid sequence MCSMYGICK [SEQ ID NO: 2], or substitutions of the sequence which retain inhibitory activity.

11. The inhibitor according to claim 7, wherein the inhibitor comprises a peptide including the amino acid sequence LCAFYIMAK [SEQ ID NO: 1], or substitutions of the sequence which retain inhibitory activity.

12. The inhibitor according to claim 8, wherein the inhibitor comprises a peptide including the amino acid sequence CAFYI [SEQ ID NO: 3], or substitutions of the sequence which retain inhibitory activity.

13. The inhibitor according to any one of claims 1 to 12, wherein the inhibitor comprises a peptide mimetic.

14. A complex comprising:
    (a) $p34^{cdc2}$ or a homolog thereof; and
    (b) an inhibitor of $p34^{cdc2}$ cell cycle regulatory kinase activity, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin, and wherein the complex substantially lacks kinase activity.

15. The complex according to claim 14, wherein the homolog is $p33^{cdk2}$.

16. A complex comprising:
    (a) human papillomavirus E7 protein; and
    (b) an inhibitor of human papillomavirus E7 protein, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of a protein selected from the group consisting of a retinoblastoma protein, p107 and a cyclin.

17. The complex according to any one of claims 14 to 16, wherein th inhibitor is derived from $p34^{cdc2}$ binding domain of a retinoblastoma protein.

18. The compl x according to any one of claims 14 to 16, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of p107.

19. The complex according to any one of claims 14 to 16, wherein the inhibitor is derived from a $p34^{cdc2}$ binding domain of cyclin E.

20. The complex according to claim 17, wherein th inhibitor is d riv d from the alpha-helix II domain of a retinoblastoma protein.

21. The complex according to claim 18, wherein the inhibitor is derived from the alpha-helix II domain of p107.

22. The complex according to claim 19, wherein the inhibitor is derived from the alpha-helix domain of cyclin E.

23. The complex according to claim 20, wherein the inhibitor comprises a peptide including the amino acid sequence MCSMYGICK [SEQ ID NO: 2], or substitutions of the sequence which retain inhibitory activity.

24. The complex according to claim 20, wherein the inhibitor comprises a peptide including the amino acid sequence LCAFYIMAK [SEQ ID NO: 1], or substitutions of the sequence which retain inhibitory activity.

25. The complex according to claim 21, wherein the inhibitor comprises a peptide including the amino acid sequence CAFYI [SEQ ID NO: 3], or substitutions of the sequence which retain inhibitory activity.

26. The complex according to any one of claims 14 to 25, wherein the inhibitor comprises a peptide mimetic.

27. A method of inhibiting $p34^{cdc2}$ cell cycle regulatory kinase activity, or the kinase activity of a homolog thereof, comprising:
(a) providing an inhibitor according to any one of claims 1 to 13; and
(b) contacting $p34^{cdc2}$ or the homolog thereof with an inhibiting amount of the inhibitor.

28. A method of inhibiting human papillomavirus E7 activity, comprising:
(a) providing an inhibitor according to claim 3 or any one of claims 4 to 13 when dependent on claim 3; and
(b) contacting human papillomavirus E7 with an inhibiting amount of the inhibitor.

29. An inhibitor according to claim 3 or any one of claims 4 to 13 when dependent on claim 3, for use in inhibiting human papillomavirus E7 activity.

30. A pharmaceutical composition comprising an inhibitor according to any one of claims 1 to 13.

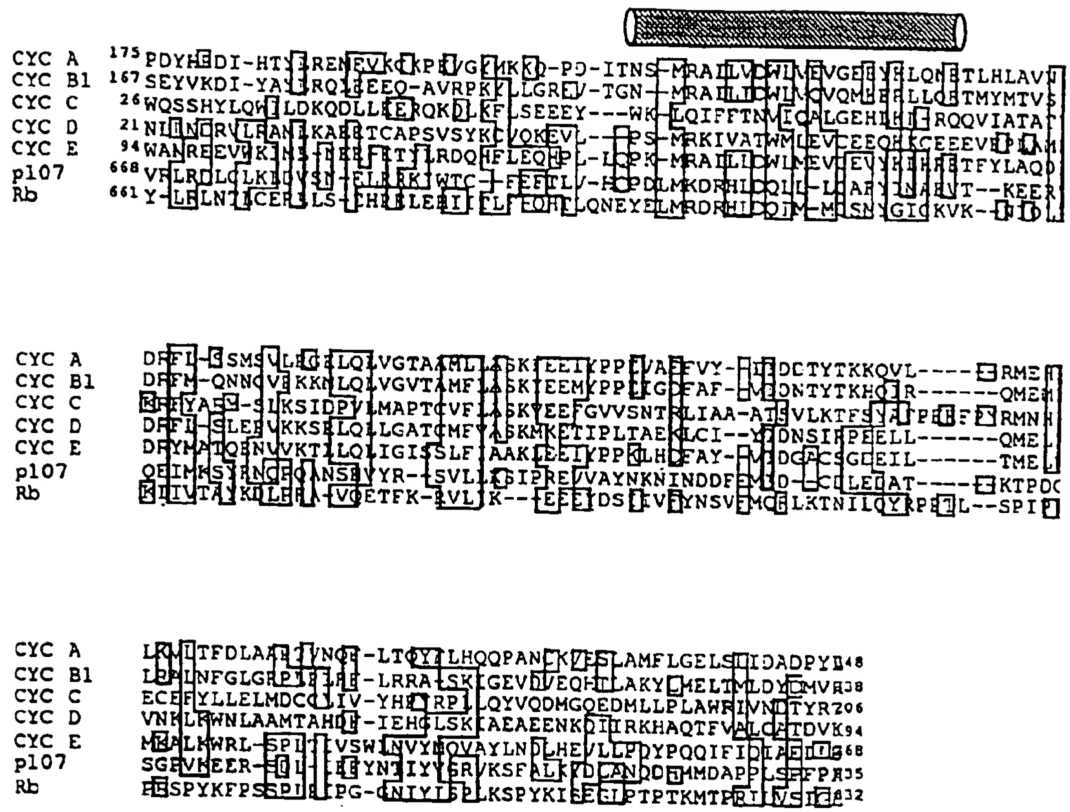

Figure 1

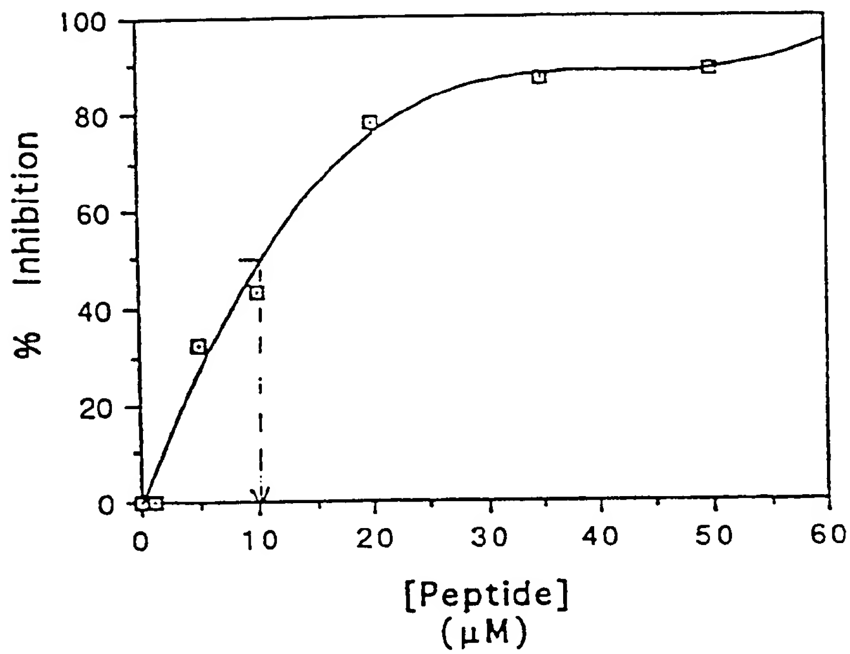

Figure 2

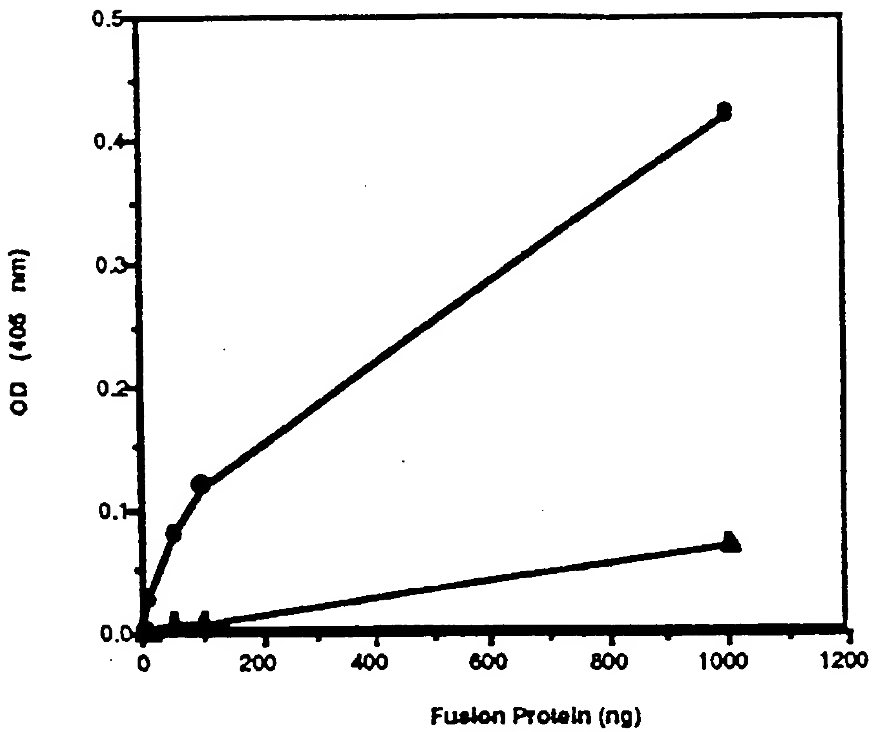

Figure 3A

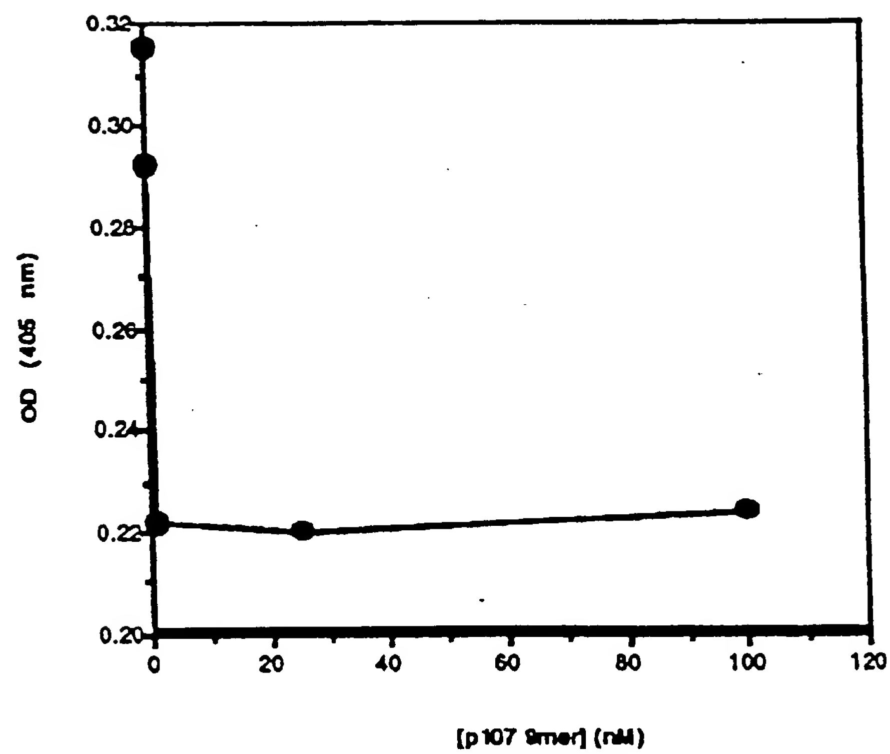

Figure 3B

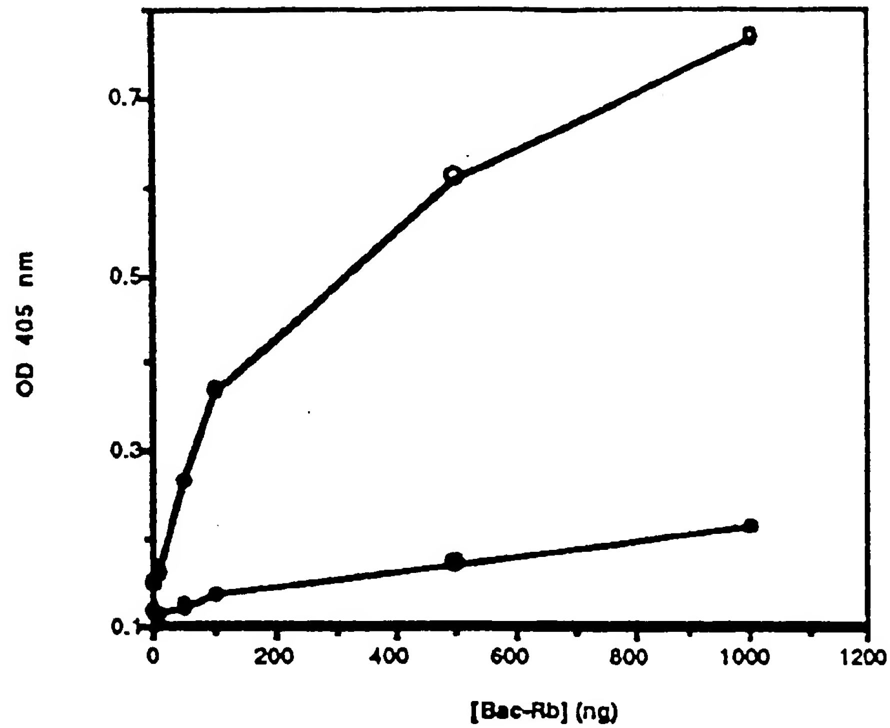

Figure 4A

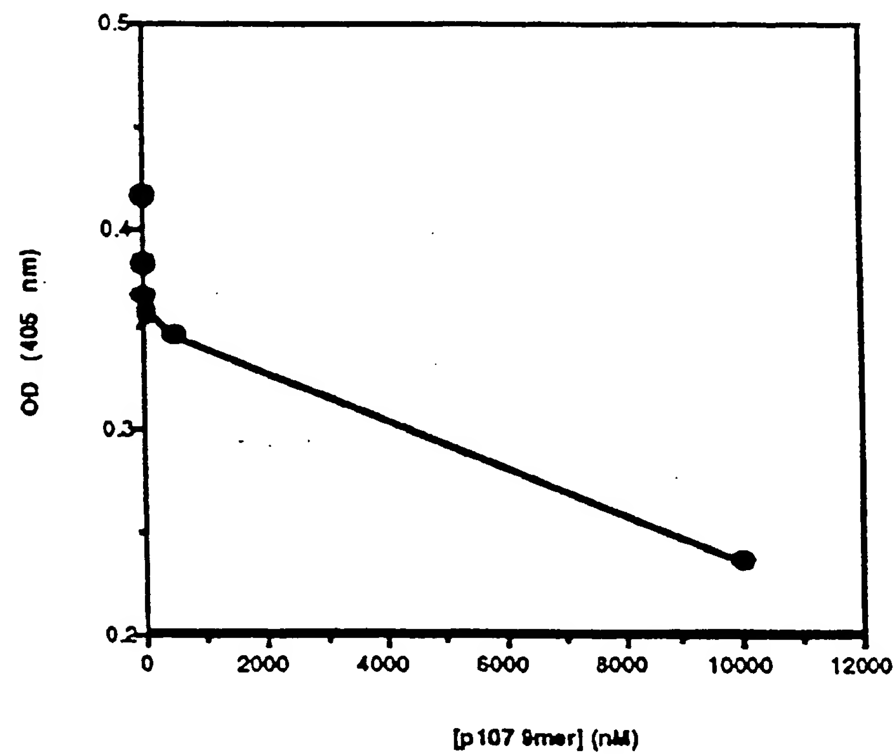

Figure 4B